Remarks

No new matter has been entered by way of this Amendment. Applicants have amended the specification to correct a typographical error in the filing date of a priority application, to include sequence identifiers, to update the status of trademarks, and to direct the entry of this corrected Sequence Listing at the end of the application. Support for the addition of sequences to the sequence listing is found in the specification at page 61, line 31; page 62, lines 2-4, 13, 15, and 28-29; page 63, line 7; and page 72, lines 6 and 11.

In compliance with 37 C.F.R. § 1.825(a), Applicants submit substitute sheets to amend the paper copy of the Sequence Listing.

In accordance with 37 C.F.R. § 1.825(a) and (b), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same and contain no new matter.

It is respectfully believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Version with markings to show changes made

In the Specification:

The paragraph beginning on page 1, line 7 was replaced with the following paragraph:

This application is a continuation-in-part of Appl. No. 09/016,361, filed January 30, 1998, which is incorporated herein by reference; and is a continuation-in-part of Appl. No. 09/098,584, filed June 17, 1998; and is a continuation-in-part of Appl. No. 09/017,735, filed February 3, 1998; and is a continuation-in-part of Appl. No. 08/589,108, filed January 23, 1996; and is a continuation-in-part of Appl. No. 08/205,713, filed March 4, 1994; and is a continuation-in-part of 08/821,739, filed March 20, 1997; and claims the benefit of U.S. Provisional Appl. No. 60/141,422, filed June 29, 1999; and claims the benefit of U.S. Provisional Appl. No. 60/170,448, filed December 13, 1999; said 08/205,713 is a continuation-in-part of 08/159,184, filed November 29, 1993, abandoned; which is a continuation-in-part of Appl. No. 08/073,205, filed June 4, 1993 , abandoned; which is a continuation-in-part of Appl. No. 08/027,146, filed March 5, 1993, abandoned; said 08/821,739 claims the benefit of U.S. Provisional Appl. No. 60/013,833, filed March 21, 1996; and said 08/821,739 is a continuation-in-part of U.S. Appl. No. 08/589,107, filed January 23, 1996 ~~July 12, 1996~~, abandoned; and is a continuation-in-part of U.S. Appl. No. 08/451,913, filed May 26, 1995, abandoned; and is a continuation-in-part of U.S. Appl. No. 08/347,610, filed December 1, 1994; and is a continuation-in-part of U.S. Appl. No. 08/186,266, filed January 25, 1994, U.S. Patent No. 5,662,907; and is a continuation-in-part of U.S. Appl. No. 08/159,339, filed November 29, 1993, U.S. Patent No. 6,037,135; which is a continuation-in-part of U.S. Appl. No. 08/103,396, filed August 6, 1993,

abandoned; which is a continuation-in-part of U.S. Appl. No. 08/027,746, filed March 5, 1993, abandoned; said 09/016,361 claims the benefit of U.S. Provisional Appl. No. 60/036,696, filed January 31, 1997, which is incorporated herein by reference.

The paragraph beginning on page 6, line 20 was replaced with the following paragraph:

Figure 1 depicts that PADRE® promotes antigen specific T cell responses from human PBMC. In Figure 1, PBMC from three healthy donors (donors 431, 397, and 344) were stimulated *in vitro*. In brief, Ficoll-Paque® (Pharmacia LKB) purified PBMC were plated at 4 x 10^6 cells/well in a 24-well tissue culture plate (Costar). The peptides were added at a final concentration of 10 μg/ml and incubated at 37°C for 4 days. Recombinant interleukin-2 was added at a final concentration of 10 ng/ml and the cultures were fed every three days with fresh media and cytokine. Two additional stimulations of the T cells with antigen were performed on approximately days 14 and 28. The T cells (3 x 10^5 cells/well) were restimulated with 10 μg/ml peptide using irradiated (7500 rads) autologous PBMC cells. T cell proliferative responses were determined using a ^{3}H-thymidine incorporation assay.

The paragraph beginning on page 59, line 6 was replaced with the following paragraph:

Since HLA-A2 is a species restricted molecule, the binding and functional activities of the A2 vaccine epitopes were measured *in vitro* using human molecules and cells. CTL epitopes were identified that demonstrated high or intermediate HLA-A2 binding affinity

(IC_{50} of $\leq$ 500 nM). These epitopes also bound to at least one additional member of the HLA-A2 supertype family with an $IC_{50} \leq 500$ nM. Each epitope stimulated the *in vitro* induction of a specific human CTL that recognized and lysed peptide-pulsed target cells and tumor cell lines expressing the relevant TAA. A PADRE® molecule is optionally included in the vaccine to promote the induction of long lasting CTL responses (Alexander *et al.*, *Immunologic Research*, In Press.).

The paragraph beginning on page 61, line 30 was replaced with the following paragraph:

A particularly preferred PADRE® molecule is a synthetic peptide, aKXVAAWTLKAAa (a = D-alanine, X = cyclohexylalanine) (SEQ ID NO:58), containing non-natural amino acids, specifically engineered to maximize both HLA-DR binding capacity and induction of T cell immune responses.

The paragraph beginning on page 62, line 1 was replaced with the following paragraph:

Alternative preferred PADRE® molecules are the peptides, aKFVAAWTLKAAa (SEQ ID NO:59), aKYVAAWTLKAAa (SEQ ID NO:60), aKFVAAYTLKAAa (SEQ ID NO:61), aKXVAAYTLKAAa (SEQ ID NO:62), aKYVAAYTLKAAa (SEQ ID NO:63), aKFVAAHTLKAAa (SEQ ID NO:64), aKXVAAHTLKAAa (SEQ ID NO:65), aKYVAAHTLKAAa (SEQ ID NO:66), aKFVAANTLKAAa (SEQ ID NO:67), aKXVAANTLKAAa (SEQ ID NO:68), aKYVAANTLKAAa (SEQ ID NO:69), AKXVAAWTLKAAA (SEQ ID NO:30), AKFVAAWTLKAAA (SEQ ID NO:31),

AKYVAAWTLKAAA (SEQ ID NO:32), AKFVAAYTLKAAA (SEQ ID NO:33), AKXVAAYTLKAAA (SEQ ID NO:34), AKYVAAYTLKAAA (SEQ ID NO:35), AKFVAAHTLKAAA (SEQ ID NO:36), AKXVAAHTLKAAA (SEQ ID NO:37), AKYVAAHTLKAAA (SEQ ID NO:38), AKFVAANTLKAAA (SEQ ID NO:39), AKXVAANTLKAAA (SEQ ID NO:40), AKYVAANTLKAAA (SEQ ID NO:41) (a = D-alanine, X = cyclohexylalanine).

The paragraph beginning on page 62, line 11 was replaced with the following paragraph:

In a presently preferred embodiment, the PADRE® peptide is amidated. For example, a particularly preferred amidated embodiment of a PADRE® molecule is conventionally written aKXVAAWTLKAAa-NH_2 (SEQ ID NO:70).

The paragraph beginning on page 62, line 14 was replaced with the following paragraph:

Competitive inhibition assays with purified HLA-DR molecules demonstrated that the PADRE® molecule aKXVAAWTLKAAa-NH_2 (SEQ ID NO:70) binds with high or intermediate affinity (IC_{50} ≤1,000 nM) to 15 out of 16 of the most prevalent HLA-DR molecules ((Kawashima *et al., Human Immunology* 59:1-14 (1998); Alexander *et al., Immunity* 1:751-761 (1994)). A comparison of the DR binding capacity of PADRE® and tetanus toxoid (TT) peptide 830-843, a "universal" epitope has been published (Panina-Bordignon *et al., Eur. J. Immunology* 19:2237-2242 (1989)). The TT 830-843 peptide bound to only seven of 16 DR molecules tested, while PADRE® bound 15 of 16. At least

1 of the 15 DR molecules that bind PADRE® is predicted to be present in >95% of all humans. Therefore, this PADRE® molecule is anticipated to induce an HTL response in virtually all patients, despite the extensive polymorphism of HLA-DR molecules in the human population.

The paragraph beginning on page 62, line 26 was replaced with the following paragraph:

PADRE® has been specifically engineered for optimal immunogenicity for human T cells. Representative data from *in vitro* primary immunizations of normal human T cells with TT 830-843 antigen and the PADRE® molecule aKXVAAWTLKAAa-NH_2 (SEQ ID NO:70) are shown in Figure 1. Peripheral blood mononuclear cells (PBMC) from three normal donors were stimulated with the peptides *in vitro*. Following the third round of stimulation, it was observed that PADRE® generated significant primary T cell responses for all three donors as measured in a standard T cell proliferation assay. With the PADRE® peptide, the 10,000 cpm proliferation level was generally reached with 10 to 100 ng/ml of antigen. In contrast, TT 830-843 antigen generated responses for only 2 out of 3 of the individuals tested. Responses approaching the 10,000 cpm range were reached with about 10,000 ng/ml of antigen. In this respect, it was noted that PADRE® was, on a molar basis, about 100-fold more potent than TT 830-843 antigen for activation of T cell responses.

The paragraph beginning on page 63, line 5 was replaced with the following paragraph:

Early data from a phase I/II investigator-sponsored trial, conducted at the University of Leiden (C.J.M. Melief), support the principle that the PADRE® molecule aKXVAAWTLKAAa (SEQ ID NO:58), possibly the amidated aKXVAAWTLKAAa -NH_2 (SEQ ID NO:70), is highly immunogenic in humans (Ressing *et al., Detection of immune responses to helper peptide, but not to viral CTL epitopes, following peptide vaccination of immunocompromised patients with recurrent cervical carcinoma.* Submitted (1999)). In this trial, a PADRE® molecule was co-emulsified with various human papilloma virus (HPV)-derived CTL epitopes and was injected into patients with recurrent or residual cervical carcinoma. However, because of the late stage of carcinoma with the study patients, it was expected that these patients were immunocompromised. The patients' immunocompromised status was demonstrated by their low frequency of influenza virus-specific CTL, reduced levels of CD3 expression, and low incidence of proliferative recall responses after *in vitro* stimulation with conventional antigens. Thus, no efficacy was anticipated in the University of Leiden trial, rather the goal of that trial was essentially to evaluate safety. Safety was, in fact, demonstrated. In addition to a favorable safety profile, PADRE® T cell reactivity was detected in four of 12 patients (Figure 2) in spite of the reduced immune competence of these patients.

The paragraph beginning on page 72, line 4 was replaced with the following paragraph:

Two peptides that stimulate HLA class II are also used in accordance with the invention. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAZTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine; "Z" is either tryptophan, tyrosine, histidine or asparagine; and "a" is either D-alanine or L-alanine (SEQ ID NO:29), has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. Two particularly preferred PADRE® molecules are the peptides, aKFVAAYTLKAAa-NH_2 (SEQ ID NO:71) and aKXVAAHTLKAAa-NH_2 (SEQ ID NO:72) (a = D-alanine, X = cyclohexylalanine), the latter containing a non-natural amino acid, specifically engineered to maximize both HLA-DR binding capacity and induction of T cell immune responses.

The pending sequence listing was replaced with the attached sequence listing.